In Vitro Cell. Dev. Biol. 28A:373–375, May 1992

0883-8364/92 $01.50+0.00

GENE TRANSFER IN PRIMARY CULTURES OF HUMAN HEPATOCYTES

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(Accepted 20 January 1992)

SUMMARY

Using liposomes as the mediator of DNA transfer, we were successful in the transfection of human hepatocytes isolated from surgical samples with an *E. coli* β-galactosidase gene (β-gal). A comparison of transfection efficiency showed that of the four promoters used, cytomegalovirus (CMV) promoter yielded higher transfection efficiencies than Rous sarcoma virus (RSV), Simian virus-40 (SV-40) and human alpha-1 antitrypsin (AAT) promoters. These studies represent the first report on the successful transfection of primary cultures of human hepatocytes.

Key words: human hepatocytes; viral promoters; liposome mediated DNA transfer; transfection; lipofection; gene therapy.

INTRODUCTION

Human hepatocyte cultures have recently been found to be a valuable experimental model for studying xenobiotic metabolism, toxicity, and liver diseases. The ability to transfer selected genes into primary human hepatocytes will be an important technical advance towards the understanding of the regulation of human liver-specific genes and the biological activities of the gene products. However, while there are numerous reports on the successful transfection of rodent hepatocytes, as of this writing, there are no similar reports on primary human hepatocytes.

In our laboratory, we have recently developed procedures for the isolation (5), cryopreservation (7,8) and induction of DNA synthesis by epidermal growth factor in cultured human hepatocytes (6). We report here our success in the liposome-mediated transfection of human hepatocytes with a reporter gene, the *E. coli* β-galactosidase (β-gal) gene.

MATERIALS AND METHODS

Chemicals. All chemicals were obtained from Sigma Co. (St. Louis, MO) unless otherwise indicated.

Isolation and culture of human hepatocytes. Human hepatocytes were isolated in our laboratory from surgical samples from patients undergoing upper abdominal surgery, with explicit informed consent from patients and Institutional Review Board approval. The hepatocytes were isolated using a collagenase-perfusion procedure that we have optimized for human liver samples. The procedure is described in detail elsewhere (5). The isolated human hepatocytes were cultured on rat tail collagen coated tissue culture plastic plates (60 mm diameter; Costar, Cambridge, MA).

Plasmid DNA preparation and transfection. The plasmid DNA used in the gene transfer experiments was the *E. coli* β-gal gene linked to different promoters including SV 40 (SV-40-β-gal; Pharmacia, Piscataway, NJ), human Alpha-1 antitrypsin [AAT-β-gal; a gift from Dr. K. Ponder (14)], Rous sarcoma virus [RSV-β-gal; (9)] and cytomegalovirus [CMV-β-gal; (10)]. Plasmids were grown in the HB101 strain of *E. coli* (Gibco BRL, Gaithersburg, MD). The DNA was isolated by Qiagen columns (Qiagen Inc., Studio City, CA). Eluted DNA was ethanol precipitated and resuspended in sterile TE buffer (10 m*M* TrisCl, 1 m*M* EGTA, pH 8.0) and quantified by measurement of absorbance at 260 nm. DNA transfer into human hepatocytes was performed using a liposome-mediated procedure modified from that reported by Ponder et al. (14) and that described in the Gibco BRL (Gaithersburg, MD) protocol. Briefly, cells were plated on collagen coated 60 mm diameter dishes at a density of 2×10^6 cells/plate. The cells were allowed to attach for 2 to 4 h after which media (1:1 mixture of F12 and Dulbecco's modified Eagle's medium, supplemented with 11.2 mg/l alanine, 12.8 mg/l serine, 24 mg/l asparagine, 2 g/l fatty acid poor bovine serum albumin, 0.168 mg/ml aminolevulinic acid, 5 mg/l oleic acid, 5 mg/l d,l-tocopherol, 0.393 mg/l dexamethasone, 7.9 mg/l d-thyroxine, 0.03 mg/l glucagon, 20 U/l insulin, 50 ng/ml human epidermal growth factor (Gibco, Long Island, NY), and 84 mg/l gentamicin) was changed to 3 ml of fresh medium. The liposome/DNA mixture was prepared by mixing 10 μg Lipofectin (Gibco BRL, Gaithersburg, MD) and specific amounts of DNA in 50 μl water. The mixture was incubated at room temperature for 15 min, added to the cultured hepatocytes and incubated at 37° C, 5% CO_2 for 18 to 20 h. The media was then changed to growth medium and the cells were incubated for an additional 48 h before harvesting for the evaluation of transfection efficiency. As a negative control, cells were treated identically except that no DNA was present in the transfection mixture.

Analysis of transfection efficiency. Transfection efficiency of β-gal was measured by cytochemical staining. Briefly, the cells were rinsed twice with phosphate-buffered saline (PBS), fixed for 10 min with 3.7% formaldehyde, and stained with 5-bromo-4-chloro-3-indoyl-β-galactopyranoside (x-gal, Strategene, La Jolla, CA) based on the method of MacGregor (9). Positively stained cells were easily visualized as blue cells. Approximately 5000 cells were randomly scored from each transfected plate. Results were expressed as percent positively-stained cells. Using this endpoint, negative control samples were found not to contain any positively stained cells.

RESULTS AND DISCUSSION

While DNA-mediated gene transfer in nontransformed, primary cultures of rodent hepatocytes has been reported (e.g., 1–4,11–17), similar reports with human hepatocytes are not available as of this writing. We showed here that liposome-mediated DNA transfer was effective in the transfection of human hepatocytes. Three gene

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transfer experiments were performed, each with hepatocytes prepared from a different donor. In all three experiments, successful transfection was achieved as demonstrated by the presence of β-galactosidase positive cells.

In the first experiment, we evaluated the feasibility of transfection of human hepatocytes with varying doses of CMV-β-gal. Plasmid DNA doses of 0.8 to 3 μg per plate yielded similar transfection efficiencies, ranging from 4 to 9% of the cell population (Fig. 1). Based on the results, 1.5 μg of DNA and 10 μg lipofectin were used in the subsequent experiments. In the second and third experiments, the transfection efficiency of β-gal linked to different promoters was studied. The promoters tested were CMV, AAT and RSV in the second experiment (Fig. 2 *A*) and the same promoters plus SV-40 in the third experiment (Fig. 2 *B*). In both experiments, CMV was found to yield the highest levels of β-gal positive cells. Our results are therefore similar to results obtained by Ponder et al. (14) with rat hepatocytes, indicating that CMV-promoter was the most effective in gene transfer.

It is interesting to note that of the three human hepatocyte isolations transfected with CMV-β-gal, one yielded close to 50% transfection, while the other two had transfection efficiency values of less than 10%. Subsequent experiments with more human hepatocyte isolates (data not shown) were more consistent with the lower values, suggesting that the high value was more of an exception than the rule. As virtually identical procedures and reagents were used for these three experiments, it is possible that host factors may be responsible for the unusually high transfection efficiency. We hope that the frequency of occurrence of this phenomenon and the exact nature of such host factors may be elucidated upon more experience with a higher number of human hepatocyte isolates.

Our success with gene transfer in primary culture of human hepatocytes suggests that genetic modification of the cells can be performed. It will be particularly interesting, for instance, to evaluate the hepatocyte-specific delivery system such as the asialoglycoprotein-polycation DNA complex as proposed by Wu and Wu (18) in the human hepatocytes.

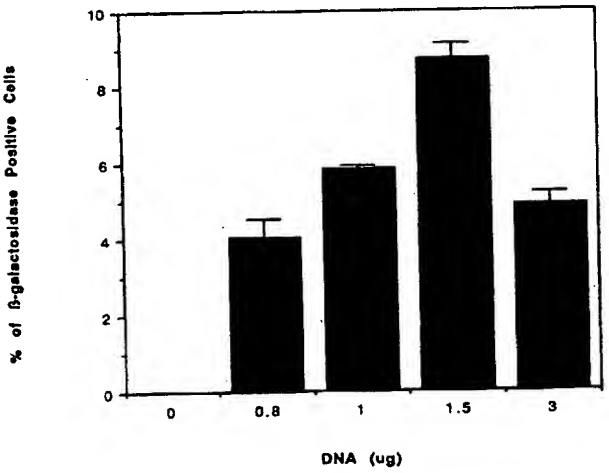

FIG. 1. Transfection efficiency of cytomegalovirus promoter-linked *E. coli* β-galactosidase DNA in human hepatocytes as a function of total DNA used. The hepatocytes were obtained from human donor 1.

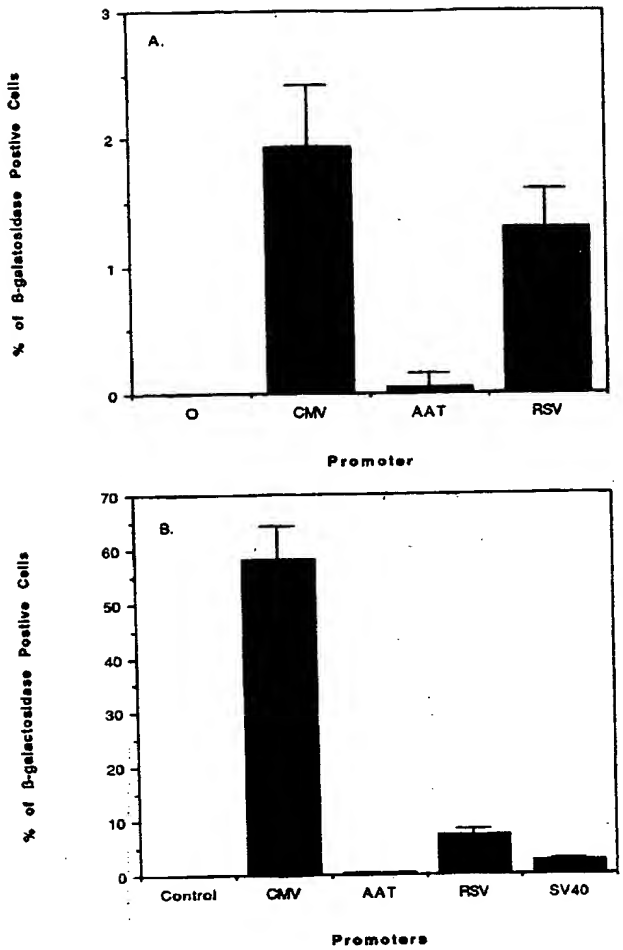

FIG. 2. A comparison of different promoters on transfection efficiency. The promoters used were: cytomegalovirus (CMV), human alpha-1 antitrypsin (AAT), Rous sarcoma virus (RSV), and Simian virus-40 (SV-40). Hepatocytes from two different individuals (human donor 2: *A*; human donor 3: *B*) were used.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. K. Ponder for the provision of plasmid DNA samples and for technical discussion on DNA transfection of hepatocytes. We also thank Dr. P. Hippomyer for his technical advice on the evaluation of β-gal positive hepatocytes.

REFERENCES

1. Chang, D. C.; Gao, P. Q.; Maxwell, B. L. High efficiency gene transfection by electroporation using a radio-frequency electric field. Biochim. Biophys. Acta 1092:153–160; 1990.
2. Fang, X. J.; Keating, A.; de-Villiers, J., et al. Tissue-specific activity of heterologous viral promoters in primary rat hepatocytes and Hep G2 cells. Hepatology 10:781–787; 1989.

3. Friedmann, T.; Xu, L.; Wolff, J., et al. Retrovirus vector-mediated gene transfer into hepatocytes. Mol. Biol. Med. 6:117–125; 1989.
4. Ginot, F.; Decaux, J. F.; Cognet, M., et al. Transfection of hepatic genes into adult rat hepatocytes in primary culture and their tissue-specific expression. Eur. J. Biochem. 180:289–294; 1989.
5. Li, A. P.; Roque, M. A.; Beck, D. J., et al. Isolation and culturing of hepatocytes from human livers. J. Tissue Cult. Meth. In press.
6. Li, A. P.; Myers, C. A.; Roque, M. A., et al. Epidermal growth factor induction of DNA synthesis in primary cultures of human hepatocytes. In Vitro Cell. Dev. Biol. 27A:831–833; 1991.
7. Loretz, L. J.; Wilson A. G. E.; Li, A. P. Promutagen activation by freshly isolated and cryopreserved rat hepatocytes. Environ. Mol. Mutag. 12:335–341; 1988.
8. Loretz, L. J.; Li, A. P.; Flye, M. W., et al. Optimization of cryopreservation procedures for rat and human hepatocytes. Xenobiotica 19:489–498; 1989.
9. MacGregor, G. R.; Caskey, C. T. Construction of plasmids that express E. coli β-galactosidase in mammalian cells. Nucleic Acid Res. 17:2365; 1989.
10. MacGregor, G. R.; Mogg, A. E.; Burke, J. F., et al. Histochemical staining of clonal mammalian cell lines expressing E. coli β-galactosidase indicate heterogenous expression of the bacterial gene. Somatic Cell Mol. Genet. 13:253–265; 1987.
11. Muakkassah-Kelly, S. F.; Jans, D. A.; Lydon, N., et al. Electroporation of cultured adult rat hepatocytes with the c-myc gene potentiates DNA synthesis in response to epidermal growth factor. Exp. Cell Res. 178:296–306; 1988.
12. Nairn, A.; Willett, B.; Grant, M. H., et al. Maintenance of differentiated function in cultured rat hepatocytes immortalized by transfection with viral DNA. Biochem. Soc. Trans. 18:1201–1202; 1990.
13. Pasco, D. S.; Fagan, J. B. Efficient DNA-mediated gene transfer into primary cultures of adult rat hepatocytes. DNA 8:535–541; 1989.
14. Ponder, K. P.; Dumbar, R. P.; Wilson, D. R., et al. Evaluation of relative promotor strength in primary hepatocytes using optimized lipofection. Human Gene Therapy 2:41–52; 1991.
15. Rippe, R. A.; Brenner, D. A.; Leffert, H. L. DNA-mediated gene transfer into adult rat hepatocytes in primary culture. Mol. Cell. Biol. 10:689–695; 1990.
16. Thompson, K. S.; Towle, H. C. Localization of the carbohydrate response element of the rat L-type pyruvate kinase gene. J. Biol. Chem. 266:8679–8682; 1991.
17. Woodworth, C. D.; Isom, H. C. Transformation of differentiated rat hepatocytes with adenovirus and adenovirus DNA. J. Virol. 61:3570–3579; 1987.
18. Wu, G. Y.; Wu, C. H. Delivery systems for gene therapy. Biotherapy 3:87–95; 1991.